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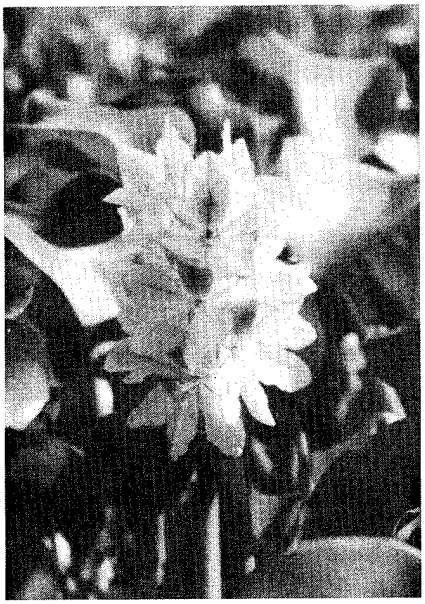
Waterhyacinth phenological control point demonstration using four herbicides

by
John D. Madsen, Chetta S. Owens, and Kurt D. Getsinger

Waterhyacinth (*Eichhornia crassipes* (Mart.) Solms), an introduced floating aquatic plant, is well established in tropical and subtropical regions of the world. This plant spreads by rapid and aggressive vegetative reproduction of daughter plants to form dense mats of vegetation that can cover thousands of hectares of an infested water body. The dense mats produced by waterhyacinth can effectively block a waterway to navigation, degrade the habitat and water quality of an aquatic ecosystem, provide a breeding ground for mosquitoes, and destroy recreational and fishery usage (Gallagher and Haller 1990; Madsen, Luu, and Getsinger 1993; Rai and Munshi 1979).



The showy inflorescence of waterhyacinth was imported to the United States at the turn of the century for use in water gardens

In the United States, waterhyacinth has a northern limit on its range due to the plant's inability to withstand low air temperatures (Aurand 1982, Tyndall 1982). In regions where the winters are severe (extended periods of temperatures below 0 °C), waterhyacinth cannot survive. However, in most of the Gulf Coast states and California, waterhyacinth has become established as an aquatic nuisance weed (Aurand 1982, Penfound and Earle 1948, Tyndall 1982).

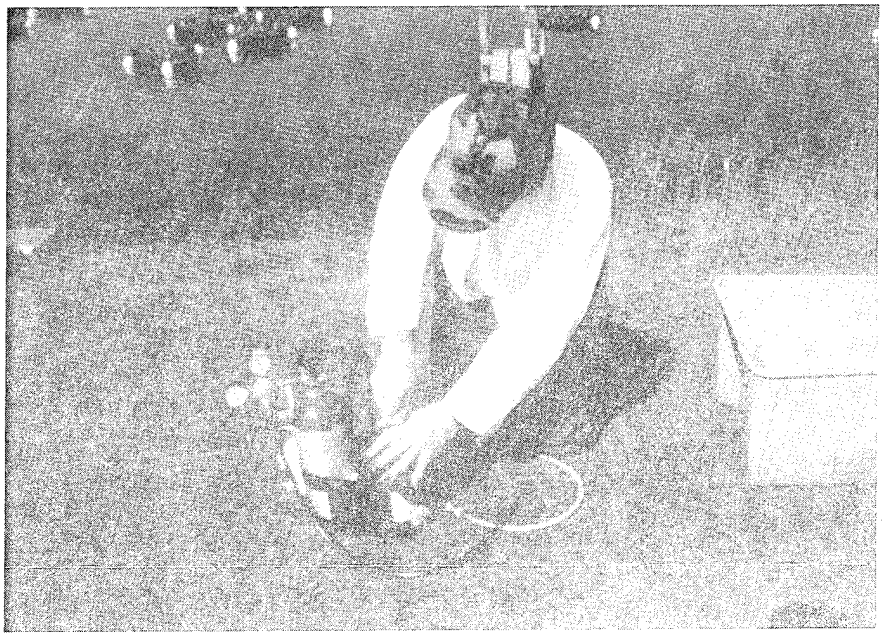
A variety of control methods are available for waterhyacinth management purposes, including mechanical, physical, biological, and chemical technologies. Chemical control techniques employ herbicides with different mechanisms of action, and are therefore applied with product-specific application rates and environmental usage considerations.

Phenology (the study of the life cycle of the target plant resulting from changes in climate or environmental conditions) provides information that can be used to maximize management strategies. From previous studies on the phenology of waterhyacinth, weak points in the life cycle were determined which could be applicable for improving control (Luu and Getsinger 1988, Madsen 1991). This article focuses on the timing of chemical applications [early (June) versus late (August)] based on the phenology of waterhyacinth and compares the efficacy of the registered aquatic herbicides glyphosate, 2,4-D, diquat, and triclopyr, currently under an Experimental Use Permit for aquatic application at the lowest recommended use rates.

Methods

This demonstration was conducted at the Lewisville Aquatic Ecosystem Research Facility, in Lewisville, Texas, during 1993. Two ponds (0.3 ha) were used, each containing 52 growth containment

rings located in water at approximately a 1-m depth. The rings (1-m^2 surface area) were constructed of wire mesh with floats attached to provide buoyancy and were fixed to an anchored cable to stabilize their initial position in the pond. Ten medium-sized adult plants (minimum of 5 adult leaves, approximately 45 cm in height) were placed in each of the 52 rings in May, a month prior to the first herbicide treatment. At this time, each pond was fertilized with 11.4 kg of ammonium sulfate to provide adequate waterhyacinth nutrition, and Aquashade was maintained at 1 mg/L in the pond for the control of planktonic algae. The ammonium sulfate was thereafter added weekly, and the Aquashade application was repeated monthly. The waterhyacinths were visually rated on a weekly basis (pre- and posttreatment) for plant vigor (good, chlorotic, or necrotic) and height.



Applicator prepares the CO_2-pressurized herbicide spraying apparatus

A timed herbicide application produced 10 ml volume per square meter (equivalent to approximately 2 qt herbicide formulation per acre)

Before the early herbicide application in June, the plants were small (average 45 cm in height) and clearly in the invasion-colonization stage of their life cycle. By the late herbicide application in August, the untreated plants had grown to an average height of 109 cm and had developed into a mature stand of waterhyacinth (Madsen 1993).

On June 14 (early treatment) and August 30 (late treatment), 1993, 40 randomly selected rings per pond were dosed with the appropriate herbicide tank mix (using a CO_2-pressurized spray system timed to deliver 10 ml per square meter) at the rates listed in Table 1. The lower range of recommended application rates was selected to maximize the importance of application timing and to demonstrate that maximum label rates are not always required to effectively control waterhyacinth.

Table 1. Application Rates for Herbicides Used in the Waterhyacinth Demonstration Project

Trade Name	Active Ingredient	Application Rate
Reward	Diquat	5.44 L + 936 L of H_2O/ha (2 qt + 100 gal of H_2O/acre)
Rodeo	Glyphosate	6.18 L + 936 L of H_2O/ha (2.5 qt + 100 gal of H_2O/acre)
Weedar 64	2,4-D	5.44 L + 936 L of H_2O/ha (2 qt + 100 gal of H_2O/acre)
Garlon 3A	Triclopyr	5.44 L + 936 L of H_2O/ha (2 qt + 100 gal of H_2O/acre)

The surfactant X-77 was added to each tank mixture at 0.25 percent volume:volume.

The early and late untreated reference waterhyacinth rings were harvested the day before treatment to provide an estimate of pretreatment biomass. Posttreatment harvests including the untreated reference were conducted at 21, 42, and 112 days.

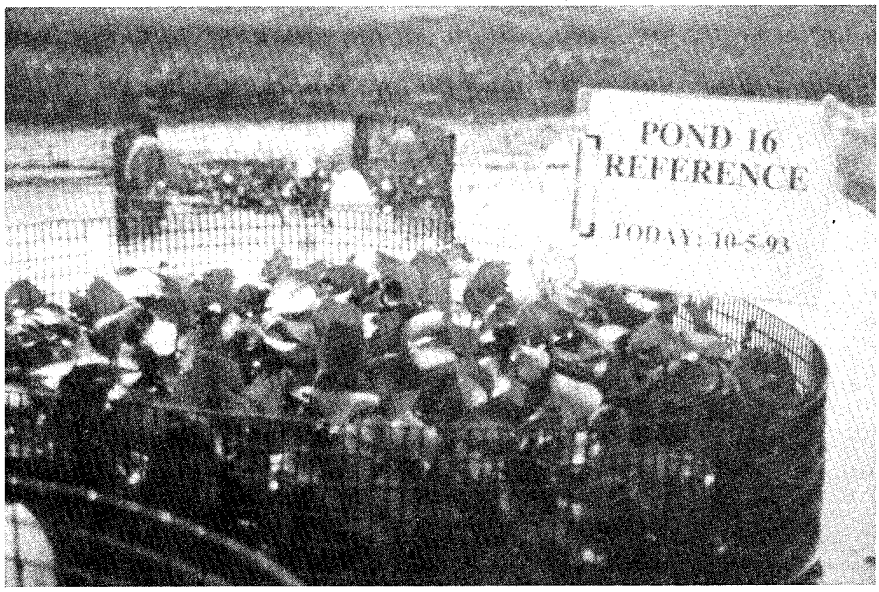
Plants within the 1-m^2 rings were removed and processed. However, for the final harvest, plants were removed within 0.25-m^2 quadrats randomly placed within the 1-m^2 ring to reduce sorting time. After each harvest, plants were separated into leaves (or aerial), stem bases, roots, and dead material. All inflorescences and daughter plants were counted for each replicate, then added to the aerial portion of the sample. Four replicate harvests were taken per treatment. Samples were oven dried at 55 °C to determine dry weight.

Data were analyzed between treatments using analysis of variance and the least significant difference test at the p = 0.05 level for means on a given sampling date.

Results and discussion

Following the early spraying, all herbicides used in this study significantly reduced total biomass of waterhyacinth at all harvest dates compared to the untreated reference (Figure 1). In contrast, late spraying did not significantly reduce biomass. This poor control following the late-season application was likely due to increased density and size of the waterhyacinth mats.

Previous phenological studies have found that although the biomass of the waterhyacinth stembase makes up a small proportion of the total plant mass, it contains a high concentration of starch, with total nonstructural carbohydrate levels varying from 10 to 40 percent depending on season (Madsen, Luu, and Getsinger 1993). The stembase of waterhyacinth



Reference waterhyacinth ring after 4 months of growth

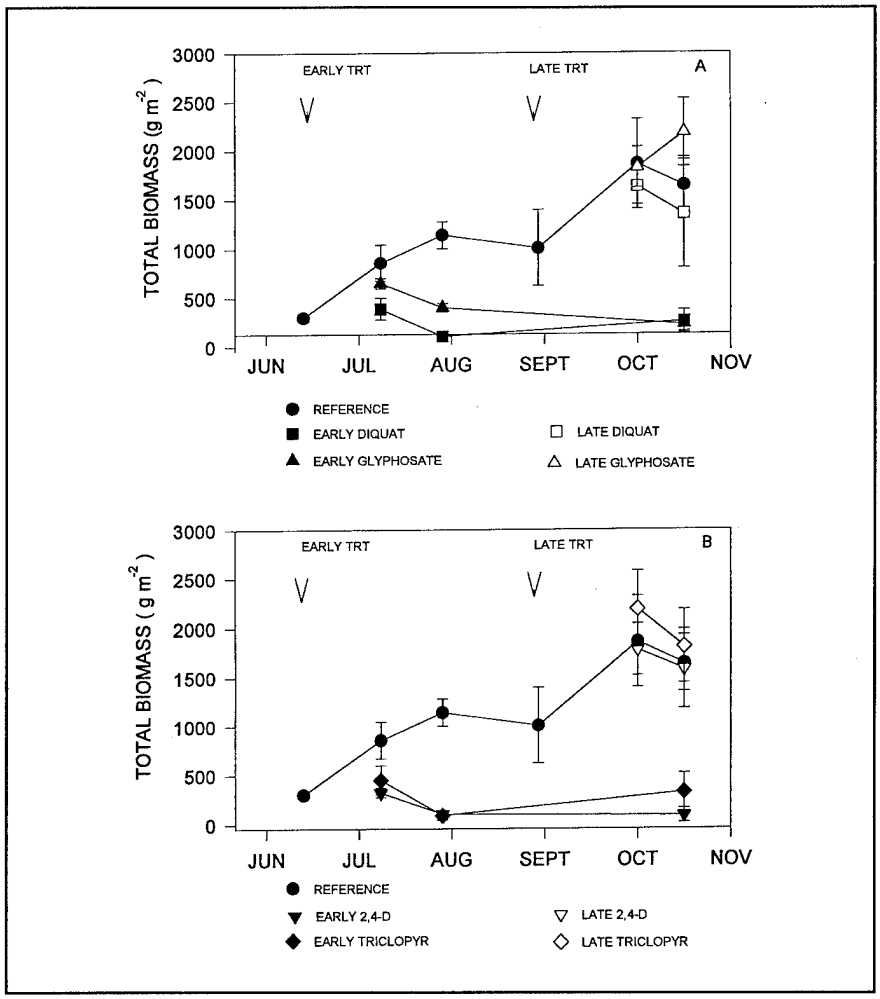
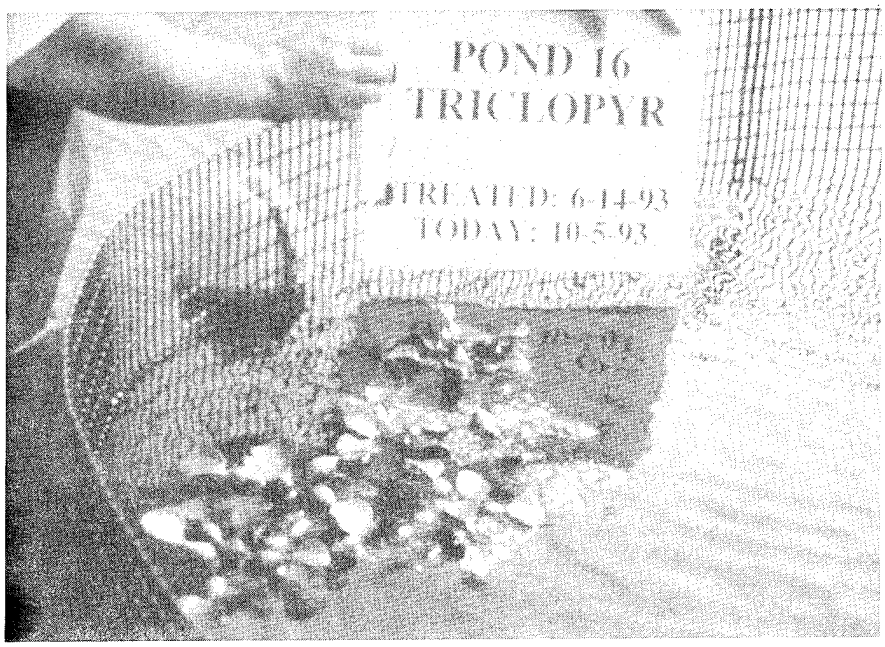


Figure 1. Total biomass of waterhyacinth reference ring and plants treated in June (early) and August (late) with (a) diquat and glyphosate and (b) 2,4-D and triclopyr. Bars indicate ±1 standard error of the mean



Waterhyacinth treated with triclopyr early in the spring showed little regrowth even by the end of the growing season

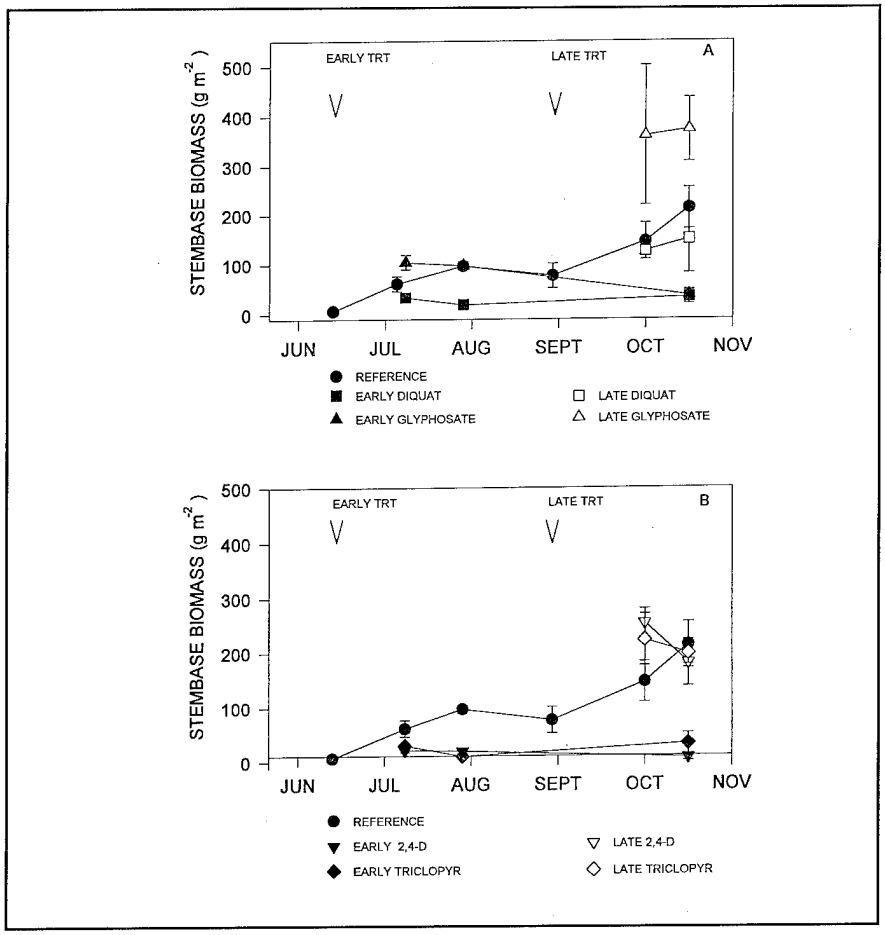


Figure 2. Stembase biomass of waterhyacinth reference ring and plants treated in June (early) and August (late) with (a) diquat and glyphosate and (b) 2,4-D and triclopyr. Bars indicate ±1 standard error of the mean

performs an important function by storing a ready chemical energy source for future growth and survival (Luu and Getsinger 1988). When herbicide-treated stembase data were compared to untreated reference data, it was found that early spraying significantly reduced stembase biomass by the final harvest date, thereby reducing the reserve capacity of the plant. Late spraying with all four herbicides did not significantly reduce stembase biomass (Figure 2). Thus, the plant could recover from the treatment stress.

The final harvest produced similar results between total biomass and stembase biomass for all treatments (Figure 3). For the total and stembase biomass, the early treatment (June) resulted in less than 21 percent regrowth as compared to the reference, and significant differences for the stembase biomass herbicide treatments versus untreated references. The late treatment (August) for the final harvest did not significantly reduce the total biomass of waterhyacinth, and plant biomass was higher for the glyphosate late application although not significantly from the reference. Stembase biomass was equal to or less than reference for diquat, 2,4-D, and triclopyr late treatments. Glyphosate, however, had significantly higher biomass from the reference and the other herbicide treatments. Further research needs to be performed to determine if this was an actual stimulatory effect by glyphosate or an experimental artifact.

Triclopyr and 2,4-D, both systemic, fast-acting herbicides, provided good control when applied to young, actively growing plants (Figure 3a). In this study, when triclopyr and 2,4-D were applied in the spring (June), the optimum time based on previous phenology data (Luu and Getsinger 1988), waterhyacinth did not significantly regrow through 4 months posttreatment. When these herbicides were sprayed in August, after the

waterhyacinth had achieved maturity, final growth was not significantly different from the reference plants at 3 months posttreatment.

Diquat, a contact herbicide, also demonstrated effective control against young waterhyacinth plants (Figure 3). By using this herbicide on young waterhyacinth, the active ingredient contacted all parts of the small plant, providing good control. When applied later in the summer, control was minimal due to the density and height of the waterhyacinth canopy which prevented the herbicide from contacting all aerial parts of the plant.

Glyphosate, a slow-acting systemic herbicide, also demonstrated good control of waterhyacinth when sprayed early in the season when plants were in a small, invasive portion of their life cycle (Figure 3). When sprayed late in the growing season there was no reduction in the total biomass of the plant, and stembase biomass was significantly greater than the reference.

Results of this demonstration show that the timing of spraying is of equal importance to herbicide selection and application rate for providing adequate control of waterhyacinth. Under proper growth stage conditions, even the lowest recommended rate of herbicide application can provide good control of the plant throughout the year.

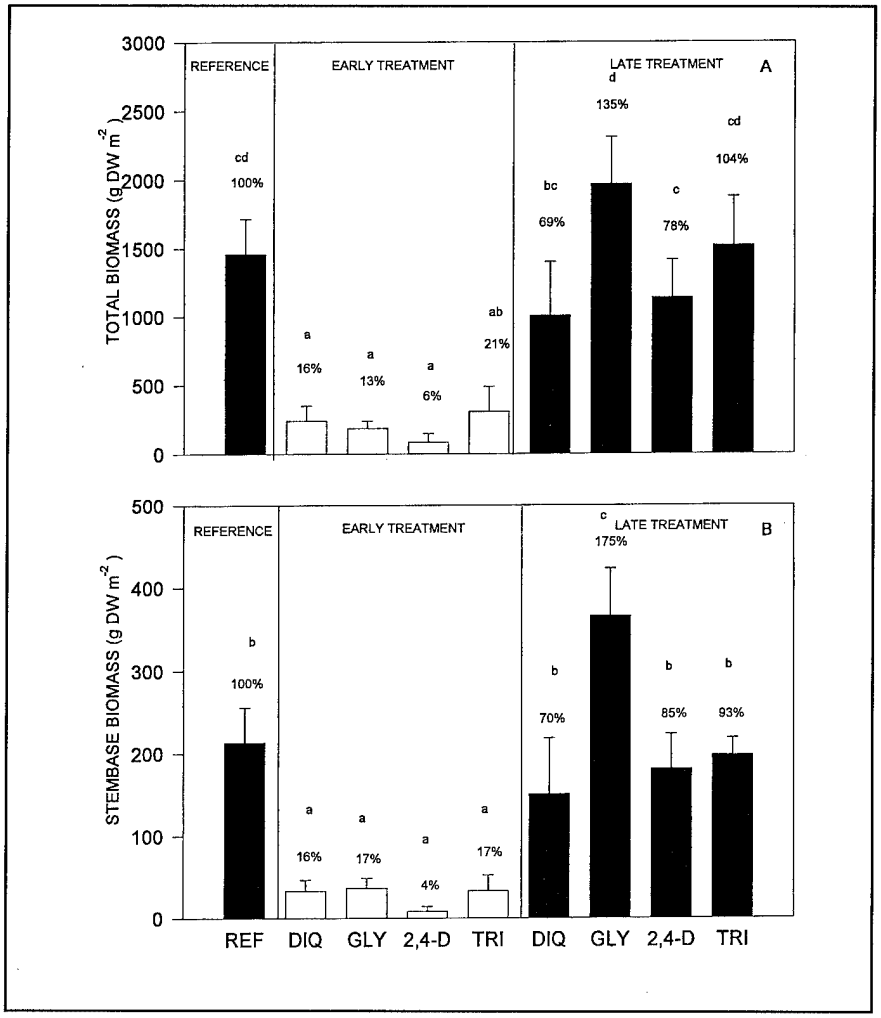


Figure 3. Total biomass (a) and stembase biomass (b) for final harvest for all treatments. Bars indicate ±1 standard error of the mean; letters indicate significant difference at p = 0.05 using least significant difference and analysis of variance

Conclusions

By understanding the phenology of waterhyacinth and utilizing the weak points in the life cycle of the plant, a better chemical management strategy can be achieved. Four commonly used aquatic herbicides exhibited good control when applied early in the growing season to actively growing plants. In fact, no significant difference in effectiveness between these four herbicides (2,4-D, diquat, glyphosate, and triclopyr) was observed 112 days after the early treatment. The late treatment date did not exhibit significant reduction in plant growth 42 days after treatment, compared to reference levels. While three of the herbicides exhibited a marginal, insignificant decrease, plant growth was actually higher for the glyphosate late application. For a management strategy, it is best to control waterhyacinth when plants are small, before a tall, mature, dense mat is formed. Maintenance control programs (Joyce 1991), such as those employed operationally in Florida, clearly demonstrate the effectiveness of this strategy.

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Dr. John D. Madsen, *a research biologist in the Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station (WES), is stationed at the Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, Texas. He conducts research on the phenology, ecology, and management of aquatic plants. Dr. Madsen has a Bachelor of Science degree from Wheaton College and Master of Science and Doctor of Philosophy degrees in Botany from the University of Wisconsin-Madison.*

Ms. Chetta S. Owens, *a research assistant for AScI Corporation of McLean, Virginia, is stationed at the LAERF, where she assists in research on the phenology and management of aquatic plants. Ms. Owens has Bachelor of Science and Master of Science degrees in Biology from Texas Woman's University.*

Dr. Kurt D. Getsinger *is a research biologist in the Environmental Laboratory, WES, and is the leader of the Chemical Control Technology Team. His expertise includes management of nuisance aquatic vegetation using herbicides and plant growth regulators. Dr. Getsinger holds a Bachelor of Science degree in Biology from Campbell University, a Master of Science degree in Biology from East Carolina University, and a Ph.D. in Plant Physiology from Clemson University.*

Integrated use of herbicides and pathogens for submersed plant control

by Michael D. Netherland and Judy F. Shearer

To develop effective, safe, and selective tools for the control of exotic aquatic species, the U.S. Army Corps of Engineers' Aquatic Plant Control Research Program (APCRP) supports active research in the areas of biological and chemical control.

Efforts in biocontrol research are aimed at identifying the most promising organisms (from a vast list of potential candidates) for controlling exotic species, characterizing their host specificity and efficacy in laboratory quarantine, and upon obtaining approval from the U.S. Department of Agriculture and/or the U.S. Environmental Protection Agency, releasing a host-specific organism (insect or pathogen) to reduce nuisance infestations.

Chemical control research is defined by products made available through industrial manufacturers (only six herbicides are currently registered for use in aquatic sites) and strict regulatory requirements (Federal, state, and local) that dictate which products can be applied, the treatment rates, and water use restrictions. Research emphasis is placed on reducing use rates of currently registered herbicides (by understanding hydrodynamic properties and life-cycle weak points of target plants) to decrease environmental loading of chemical active ingredients or to provide improved species-selective control.

Although the research and operational approaches to biological and chemical control are quite different, their common objectives (reducing nuisance exotic plants and promoting beneficial vegetation) suggest the potential for integrating these technologies to improve current control strategies.

From an aquatic plant management perspective, integrated control can be defined as a cost-effective, environmentally sound management system that incorporates optimal control techniques (biological, chemical, mechanical, physical) to reduce exotic plant populations to levels that cause no economic or ecological harm. This approach implies that a combination of different control measures will be used instead of an approach based on a single control measure (Murphy and Pieterse 1990). The rationale of this approach is to combine the strengths of different technologies and to reduce or eliminate inherent weaknesses of the various technologies.

In terrestrial systems the term "integrated control" is commonly associated with management techniques aimed at reducing reliance on pesticides. However, in aquatic systems, Murphy and Pieterse (1990) state that herbicides are applied only if other means of aquatic plant control are ineffective or too costly. Therefore, decreasing the use of chemicals is usually not a major objective of integrated control. This statement must be balanced against the recent emphasis placed on reducing rates and improving the timing of application of herbicides to reduce environmental loading or increase species selectivity. Herbicide use at reduced rates, or the ability to apply herbicides less frequently, will give plant managers from state and Federal agencies more flexibility in integrating chemicals into their overall plant management program. It is likely that the public will continue to demand a reduction in chemical usage and use rates. Thus, it is important to integrate control techniques to meet this demand while maintaining the ability to use herbicides to provide adequate and cost-effective plant control.

Several researchers have investigated combining chemical and biological methods for improved plant control. Herbicide and insect combinations have been applied to floating or emergent plants. Reduced rates of the herbicide 2,4-D have been combined with the alligator weed flea beetle (*Agasicles hygrophila*) or the mottled waterhyacinth weevil (*Neochetina eichhorniae*) for control of alligator weed (*Alternanthera philoxeroides*) or waterhyacinth (*Eichhornia crassipes*) (Foret, Spencer, and Gangstad 1974; Blackburn and Durden 1975; Perkins 1977). Integrated use of herbicides and grass carp (*Ctenopharyngodon idella*) has been accomplished by applying herbicides to reduce the initial biomass of the target vegetation, and subsequently stocking a low density of grass carp to maintain control (Leslie and others 1987).

Efforts to integrate herbicides with pathogens have generally been directed at submersed vegetation. Sorsa, Nordheim, and Andrews (1988) examined the effect of combining the contact herbicide endothall with the fungal pathogen *Colletotrichum* sp. for control of Eurasian watermilfoil (*Myriophyllum spicatum*). Smit and others (1990) combined the systemic herbicide fluridone with isolates of various endemic fungal species and evaluated control of coontail (*Ceratophyllum demersum*). Integrating these approaches provided improved efficacy versus either method on its own.

As noted earlier, the APCRP strongly supports independent research activities in biological and chemical control. The goal of this newly created integrated control work unit is to draw upon the significant amount of historical data from APCRP work units and use this information to combine technologies for improved control. This article describes the results of a collaborative study between the chemical and biocontrol teams using a herbicide in combination with a plant pathogen for enhanced control of a noxious submersed plant.

Biological control

Biological control under the APCRP has included identification and evaluation of host-specific microbes to provide control of exotic submersed species. Microbes can often act as weak pathogens, affecting growth and/or morphology but rarely killing the target plant. When microbial population densities are low, natural plant defense mechanisms often able to ward off the spread of disease beyond initially infected areas (due to a low number of infective units). Pathogens cultured in the laboratory and applied to a susceptible plant population at a high rate (augmenting the natural pathogen population) can overwhelm plant defense mechanisms. This approach to using plant pathogens is called the inundative method of biocontrol (Templeton, TeBeest, and Smith 1979) and has been used successfully in terrestrial systems as evidenced by the release of the mycoherbicides Collego for the control of northern joint vetch and Biomal for the control of round-leaved mallow (Harris 1993).

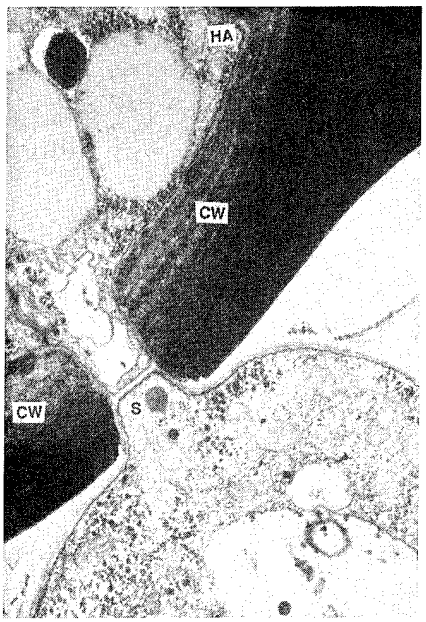
Inundative biocontrol can be applied to aquatic plant management by using a microbe(s) specific to a target aquatic macrophyte population. An APCRP work unit designed to identify microorganisms for inundative management of hydrilla (*Hydrilla verticillata*) was initiated in the mid-1980s. Hydrilla is an excellent target species for pathogen biocontrol because it is a perennial plant that forms extensive monocultures.

The fungal pathogen *Mycoleptodiscus terrestris* (MT) was isolated from hydrilla tissue collected at Lake Houston, Texas (Joye 1990). Subsequent laboratory work identified MT as a candidate for hydrilla control. As the fungus is dispersed in water, it settles on stems and leaves and quickly attaches to the plant surface. Each contact point of the fungus becomes a potential plant cell invasion site. Electron microscopy studies have confirmed that MT can directly penetrate through the cell wall, ramifying throughout tissue systems and resulting in collapse of the plant (Joye and Paul 1991).

Chemical control

Chemical control research under the APCRP has included extensive evaluation of the herbicide fluridone for controlling hydrilla. Although fluridone treatment can result in excellent long-term control of hydrilla at rates as low as 10 µg/L, an extended exposure time (60 to 90 days) is required for optimal control (Netherland, Getsinger, and Turner 1993; Fox, Haller, and Shilling 1994). Fluridone's long-term exposure requirement and the long time lag (weeks to months) between initial treatment effects and eventual plant death continue to limit its use in many aquatic systems.

Following a fluridone treatment, new plant growth displays a bleached (white) appearance. Specifically, fluridone acts by inhibiting the biosynthesis of carotenoids which function to absorb light energy to protect chlorophyll molecules from photodestruction (Bartels and Watson 1978). Chlorophyll-deficient growth is non-photosynthetic, resulting in a net demand for carbohydrates. As carbohydrate stores are depleted, mature tissue (that has sustained the plant) loses metabolic efficiency, and plants become weakened and eventually succumb to physical disturbance, herbivory, or pathogenic attack. In fact, it has been suggested that herbicides that block metabolic pathways (for example, glyphosate and fluridone), rather than killing the plant directly, can weaken defense mechanisms and operate indirectly by increasing susceptibility to traditional nonlethal agents such as pathogens (Kerfoot 1989).



Penetration of *Mycoleptodiscus terrestris* into an epidermal cell of hydrilla (HA = fungal hypha, CW = cell wall of hydrilla host cell, S = fungal septum) (photograph from Joye and Paul 1991)

In assessing the strengths and weaknesses of using either fluridone or MT alone for hydrilla control, it was noted that the individual strengths of each technology frequently offset the respective weaknesses of each technology (Table 1). This assessment led to initial discussions and planning for integrating these technologies in a laboratory-scale evaluation. The objective of this pilot study was to determine the potential additive or antagonistic effects of combining a

fluridone treatment with the plant pathogen MT for hydrilla control.

Table 1.
Comparative Strengths and Weaknesses of the Biocontrol Agent MT and the Herbicide Fluridone for Control of Hydrilla

Mycoleptodiscus terrestris	Fluridone
Strengths	**Weaknesses**
High specificity (studies still required)	Low to moderate specificity (species, rate, and timing)
Rapid results	Delayed results
Very short exposure requirement	Extended exposure requirement
Weaknesses	**Strengths**
Variable activity (temperature, virulence)	Defined dose response
Little to no field verification	Proven method of control
Little residual activity (limitation of current formulation)	No regrowth during exposure
Not currently registered for use	EPA registered

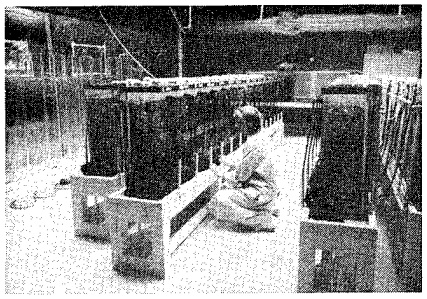
Materials and methods

To test the effects of combining fluridone and MT for hydrilla control, a study was conducted in a walk-in growth chamber previously described for herbicide concentration/exposure time studies conducted at the U.S. Army Engineer Waterways Experiment Station (WES) (Netherland, Green, and Getsinger 1991; Netherland, Getsinger, and Turner 1993). This system consists of fifty-two 55-L aquaria (0.9 m tall × 0.09 m^2) located in a controlled-environment room with a temperature of 23 ± 2 °C, light intensity (photosynthetic active radiation measured at the water surface) of 520 ± 60 µmol/ m^2/sec, and photoperiod of 14L:10D. Overhead lighting was provided by a combination of 400-W mercury vapor bulbs and 250-W high-pressure sodium lamps. A general water culture solution recommended by Smart and Barko (1984) for growing aquatic macrophytes was used throughout the study.

Hydrilla apical tips were obtained from the Suwannee River, Florida, and sediment was collected from Brown's lake at WES. Sediment was enriched with NH_4Cl (200 mg/L) to prevent nitrogen limitation during the course of the studies. Glass beakers (300 ml) were filled with sediment, and four 10- to 15-cm hydrilla apical tips were planted (5 cm deep) in each beaker. Beakers were capped with a layer of silica sand (0.5 cm) to prevent sediment suspension in the water column. Eleven beakers were placed in each aquarium, and water was exchanged (one exchange per 24 hr) during the pretreatment growth period. Air was lightly bubbled through each aquarium to provide a source of CO_2 and mixing of the water column.

A pretreatment growth period of 4 weeks resulted in the formation of a thin surface canopy and development of a viable root system. Prior to treatment, one beaker was removed from each aquarium to provide an estimate of pretreatment biomass. Estimated dry weight (DW) of shoot and root biomass remaining in each aquarium was 11.7 ± 1.3 g DW (128 g DW m^2) and 1.8 ± 0.22 g DW, respectively. Pretreatment shoot biomass for this study approximates spring to early summer biomass reported for hydrilla (Harlan, Davis, and Pesacreta 1985).

At the time of treatment, the flow-through water system was deactivated and fluridone, MT, fluridone + MT, and untreated control treatments were randomly assigned to a test aquarium resulting in 13 exposure scenarios (Table 2). Each treatment was replicated 3 times. Fluridone stock solutions were prepared from the commercial formulation Sonar AS (4 lb active ingredient per gallon). All treatment concentrations are reported as micrograms per liter (parts per billion) of the active ingredient fluridone. MT test inoculum was prepared, with the final fungal slurry having a thick consistency and a colony forming unit (CFU) count of 1×10^6 CFUs/ml. All treatment concentrations are reported as CFU/ml, and each CFU has the potential of developing into a fungal colony or disease-causing unit. MT was dispensed to the water surface with a macro-pipettor and allowed to disperse through the water column.



Environmental chamber used for laboratory testing of fluridone and *M. terrestris*

Table 2. Fluridone, MT, and Fluridone + MT Treatment Rates for Control of Hydrilla

Treatment	Rate, µg/L	Rate CFU/ml
Fluridone	2	—
Fluridone	5	—
Fluridone	12	—
MT	—	100
MT	—	200
MT	—	400
Fluridone + MT	2	100
Fluridone + MT	2	200
Fluridone + MT	5	100
Fluridone + MT	5	200
Fluridone + MT	12	100
Fluridone + MT	12	200
Untreated ref	—	—

Total chlorophyll content was measured on 4-cm apical tips at pretreatment, weekly through 28 days, and then every other week through 84 days. Three apical tips were removed from each aquarium on sampling dates, and a fresh weight was recorded. Total chlorophyll of these apical portions

was determined using a DMSO method described by Hiscox and Israelstam (1979).

Two beakers were harvested from each aquarium at 14, 28, 42, 60, and 94 days after treatment (DAT), and all aboveground shoot material was collected, washed, and dried at 70 °C for 48 hr.

Results and discussion

Results showed dramatic differences in the pattern of initial and long-term hydrilla response to fluridone, MT, and the integrated fluridone/MT treatment. For reference, untreated control plants maintained healthy growth and increased in biomass throughout the study. Moreover, chlorophyll content (measured as an indicator of physiological competence) generally remained constant in reference treatments. However, some reduction in vigor was noted toward the end of the study, indicating stress or nutrient limitation.

The chlorophyll content of treated plants allowed early prediction of the capability of hydrilla to recover from treatment. Following initial treatment injury, if plants began to show physiological recovery, biomass recovery always followed (chlorophyll recovery often occurred days to weeks ahead of biomass recovery). If however, chlorophyll levels remained significantly reduced compared to untreated controls, it indicated that further biomass loss would occur. The significant time lag between chlorophyll recovery and biomass recovery has also been noted in previous fluridone studies (Spencer and Ksander 1989; Netherland, Getsinger, and Turner 1993).

The characteristic bleaching of apical tips was noted within days following fluridone treatment. Overall, hydrilla response to fluridone was dose dependent, with significant differences in biomass (t-test 0.05) noted between treatment rates at each sample period (with the exception of 5 and 12 µg/L at 94 DAT) (Figure 1). Following an initial dose response for up to 21 DAT, chlorophyll content of the 5- and 12-µg/L treatments showed no significant differences; however, the 2-µg/L treatment remained significantly reduced throughout the study (Figure 2). This dose-dependent response has been noted in previous studies and is especially pronounced at the low fluridone rates used in this study (Netherland, Getsinger, and Turner 1993).

Percent control achieved was essentially linear over time with eventual reductions of 61, 90, and 94 percent for treatment rates of 2 µg/L (percent control = 1.0 + 0.71(x), r^2 = 0.96), 5 µg/L (percent control = 0.12 + 1.0(x), r^2 = 0.92), and 12 µg/L (percent control = 6.6 + 1.2(x), r^2 = 0.89), respectively (Figure 1). Previous studies have also resulted in the inability to completely control hydrilla following extended exposure periods to fluridone under laboratory conditions (Netherland, Green, and Getsinger 1991; Netherland, Getsinger, and Turner 1993).

It should be noted that hydrilla treated in the laboratory manifests the classic symptoms associated with fluridone (new growth has a bleached appearance). However, over time, considerable differences are observed between the integrity of laboratory- and field-treated plants. By comparison, loss of overall elasticity and vigor (plants are described as mushy) in field-treated plants is much more severe than in laboratory-treated plants. The cause for this difference in condition between the laboratory and field is not clear. However, it is likely that conditions in the field (temperature, light penetration, mechanical disturbance, herbivory, microbial attack) enhance the efficacy of fluridone. One postulate deserving further investigation is that a herbicide such

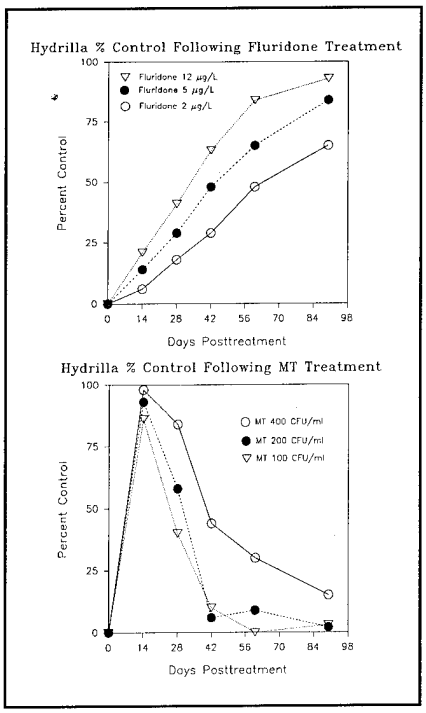


Figure 1. Percent control of hydrilla shoot biomass following fluridone and *Mycoleptodiscus terrestris* (MT) treatments. Symbols represent the average of three replicate samples

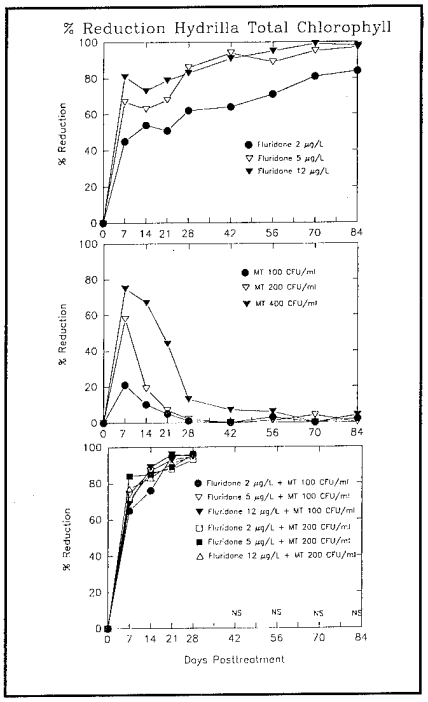


Figure 2. Percent control of hydrilla total chlorophyll following fluridone, *Mycoleptodiscus terrestris* (MT), and fluridone/MT treatments. Symbols represent the average of three replicate samples

as fluridone (metabolic blocker) may make the plant more susceptible to endemic pathogenic attack (Kerfoot 1989). The relatively sterile conditions of aquariums would likely preclude a large buildup of pathogens. Determination of pathogen response to fluridone (over time) on field and laboratory populations of hydrilla may provide some answers.

Within days following MT treatment, hydrilla leaves became translucent and began to detach from the stems. By 7 DAT, stems were nearly completely defoliated, and significant stem damage had occurred at the higher MT treatment rates of 200 and 400 CFU/ml. Initial results indicated that hydrilla responded to MT treatment in a dose-dependent manner.

Chlorophyll was significantly reduced compared to untreated controls (likely due to the significant initial defoliation) at 7 and 14 DAT; however, evidence of physiological recovery had begun by 14 DAT (Figure 2). Biomass measurements at 14 DAT indicated reductions of 97, 91, and 82 percent following MT treatments of 400, 200, and 100 CFU/ml (Figure 3). Complete chlorophyll recovery was noted for all treatments between 21 and 28 DAT (Figure 2).

Visual assessments and the harvest at 28 DAT confirmed that, although biomass was still significantly reduced compared to untreated controls, recovery had begun to occur between 14 and 28 DAT. Biomass recovered rapidly and, by 42 DAT, recovery from 100 and 200 CFU/ml treatments resulted in no significant differences compared to untreated controls (Figure 3). Although the 400 CFU/ml treatment was slow in recovering, complete hydrilla recovery occurred by the final harvest (Figure 1). The laboratory response of hydrilla to MT treatment was similar to that reported during pilot field trials (knockdown followed by rapid regrowth) conducted in ponds at Lewisville, Texas (personal communication, Michael Smart, Lewisville Aquatic Ecosystem Research Facility).

The fluridone/MT treatments resulted in initial symptoms similar to the MT treatment, with leaves becoming translucent and stems defoliated or severely injured (200 CFU/ml) within 1 week posttreatment. Chlorophyll content was significantly reduced by 7 DAT and continued to decline up to 35 DAT, at which point samples were no longer taken due to the poor condition of the plants and inadequate biomass for replicated tissue samples (Figure 2). By 14 DAT the fluridone/MT treatments were very similar to the MT treatment alone, with biomass reductions ranging from 89 to 96 percent (Figure 3). New apical shoots were sprouting from uninjured stems and root-crowns (similar to the MT treatment); however, within days, fluridone symptoms (bleached apices) were manifest and inhibited further tip growth. Harvests at 14 and 28 DAT were characterized by

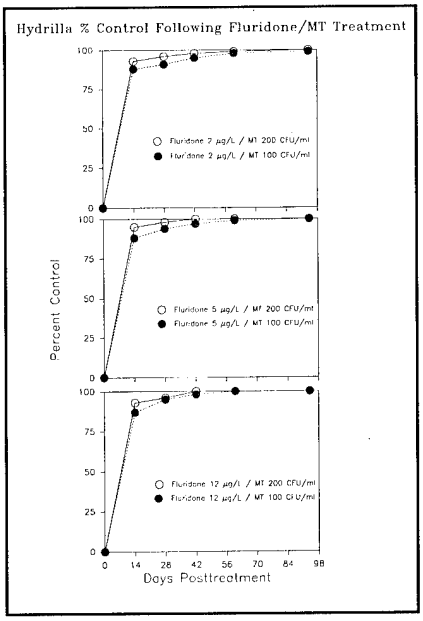
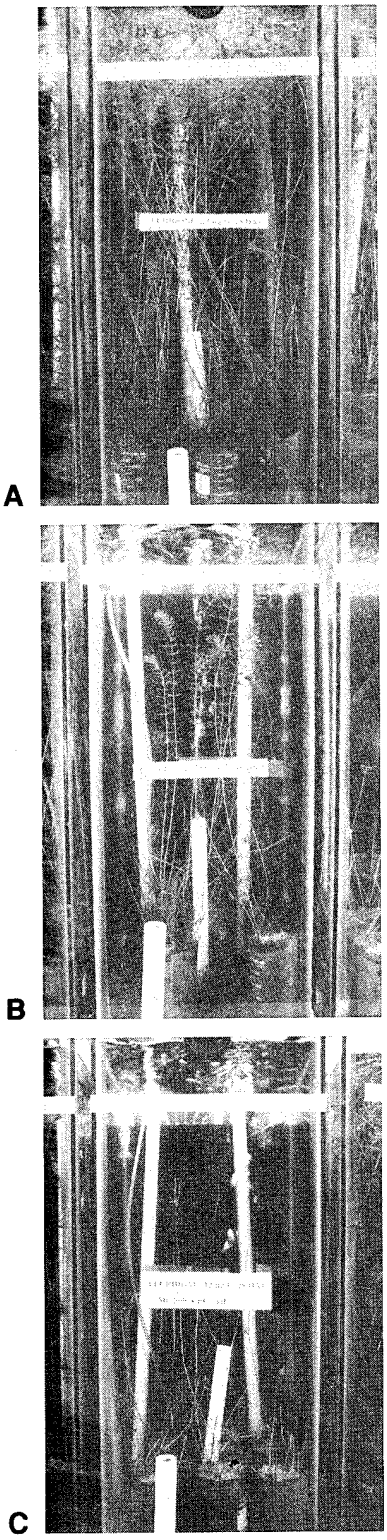


Figure 3. Percent control of hydrilla shoot biomass following an integrated fluridone/*Mycoleptodiscus terrestris* (MT) treatment at several rates. Symbols represent the average of three replicate samples



Efficacy of fluridone and *M. terrestris* treatments at 28 days posttreatment (A = fluridone at 12 µg/L, B = MT at 200 CFU/ml, C = fluridone/MT at 12 µg/L/200 CFU/ml)

hydrilla tissue which lacked integrity and showed little potential for recovery.

Complete hydrilla control was recorded at 42 and 60 DAT with the 5 and 12 µg/L + 200 and 100 CFU/ml treatments, respectively. The 2 µg/L fluridone/MT at 200 and 100 CFU/ml resulted in complete control of hydrilla by 60 to 94 DAT. The dose response noted with fluridone alone did not apply to the fluridone + MT treatment. No significant differences in percent control were noted between the 2, 5, and 12 µg/L (+MT) treatments at any sample point.

Laboratory results suggest that MT applied as a fungal mycelium acts as a contact mycoherbicide, yet lack of residual control resulted in recovery of hydrilla over time. Work on formulating MT (packaged in inert materials) to provide improved residual control is ongoing. Fluridone treatment provided good long-term hydrilla control. However, poor initial control and the requirement for extended exposure periods will continue to limit applications of this product in some aquatic systems. Research to improve delivery systems (controlled-release, metering, split applications) is also being conducted to optimize the use of fluridone.

Integrating fluridone and MT provided the benefits of excellent initial biomass reduction exhibited by MT along with long-term hydrilla control provided by fluridone. Combining these treatments greatly reduced fluridone exposure requirements while also reducing the rate of fluridone necessary to provide control of hydrilla. Of significant note was the ability of the 2 µg/L treatment in conjunction with both rates of MT to provide complete control of hydrilla. Preliminary evidence suggests that the initial injury caused by the MT stressed the hydrilla and increased susceptibility to fluridone at a rate that has not proven lethal in past studies.

These preliminary results demonstrate the potential for combining chemical and biological control agents to improve efficacy, thereby reducing reliance on chemicals (through lower use rates or less frequent applications). In addition, careful assessment of the comparative strengths and weaknesses of chemical and biological control agents provides a sound basis for combining technologies.

Future work

Future work in this area will include determination of the specificity (hydrilla and beneficial native species) of fluridone/MT at various rates and exposure scenarios. Studies will be scaled-up to the outdoor mesocosm level for validation of laboratory results and to determine the effects of such variables as temperature, water quality, light penetration, and species composition on various fluridone and MT treatment combinations. In addition, following field, mesocosm, and laboratory applications of fluridone, hydrilla will be analyzed in the laboratory to determine the qualitative and quantitative nature of endemic pathogenic assemblages associated with treatment.

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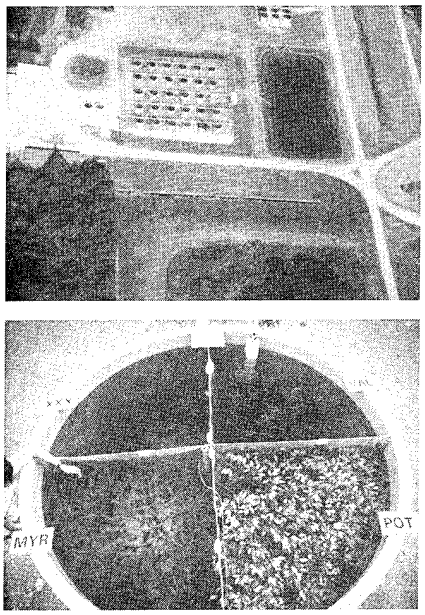

Mesocosm system and close-up of mesocosm tank that will be used to perform multispecies selectivity testing of fluridone and *M. terrestris*

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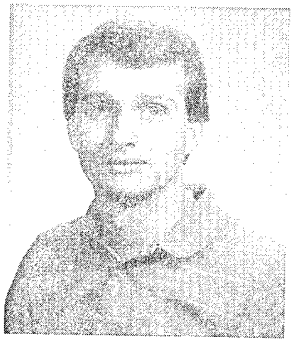

Michael Netherland *is a research biologist for the Chemical Control Technology Team in the Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station (WES). His research focuses on the effects of herbicides and plant growth regulators on nuisance submersed aquatic vegetation. Michael has a Bachelor of Science degree in Biology from Cameron University and a Master of Science degree in Botany from Purdue.*

Dr. Judy Shearer *is a research pathologist in the Environmental Laboratory, WES. She conducts research on the use of plant pathogenic fungi for the management of problem plant species. Dr. Shearer holds a Bachelor of Science degree in Political Science, a Master's degree in Botany/Mycology, and a Ph.D. in Botany/Mycology from Iowa State University.*

This issue reports on two studies. The first demonstrates the effectiveness of a phenologically based control point for waterhyacinth. Researchers evaluated the control efficiency of four aquatic herbicides—applied at the lower range of their recommended rates, at two application times. The second study examines the integrated use of biological and chemical control methods (pathogens and herbicides) for enhanced management of submersed aquatic plants.

AQUATIC PLANT CONTROL RESEARCH PROGRAM

This bulletin is published in accordance with AR 25-30 as one of the information dissemination functions of the Environmental Laboratory of the U.S. Army Engineer Waterways Experiment Station. It is principally intended to be a forum whereby information pertaining to and resulting from the Corps of Engineers' nationwide Aquatic Plant Control Research Program (APCRP) can be rapidly and widely disseminated to Corps District and Division offices and other Federal and State agencies, universities, research institutes, corporations, and individuals. Contributions are solicited, but should be relevant to the management of aquatic plants, providing tools and techniques for the control of problem aquatic plant infestations in the Nation's waterways. These management methods must be effective, economical, and environmentally compatible. The contents of this bulletin are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such products. This bulletin will be issued on an irregular basis as dictated by the quantity and importance of information to be disseminated. Communications are welcomed and should be addressed to the Environmental Laboratory, ATTN: J. L. Decell, U.S. Army Engineer Waterways Experiment Station, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, or call (601) 634-3494.

ROBERT W. WHALIN, PhD, PE
Director

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